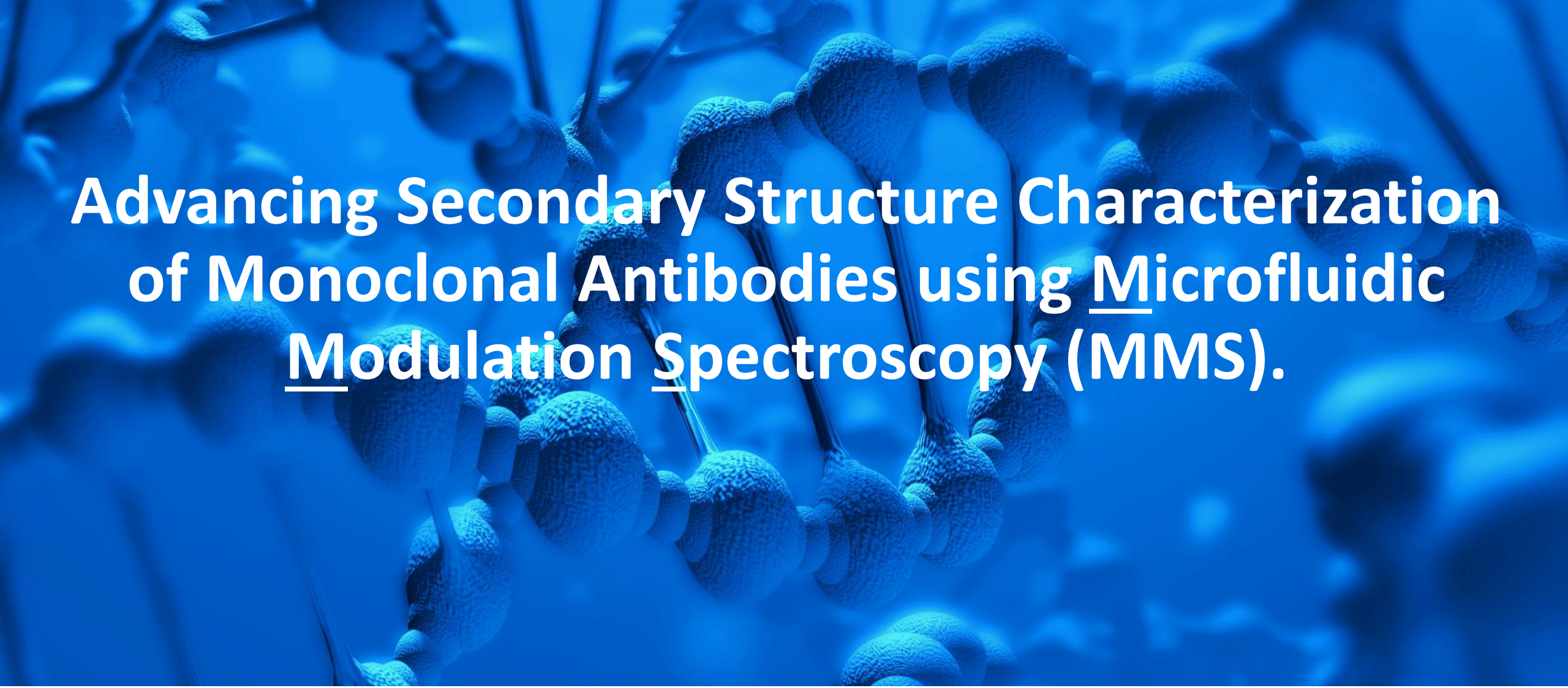

Advancing Secondary Structure Characterization of Monoclonal Antibodies using Microfluidic Modulation Spectroscopy (MMS).

DIPANWITA (DIPA) BATABYAL

PROCESS DEVELOPMENT PRINCIPAL SCIENTIST, ATTRIBUTE SCIENCES, HOS, AMGEN INC.

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Presentation Outline

- Introduction
- MMS Data
  - Method Testing (1)
  - Method Development (2)
  - Method Advancement (3)
- Conclusion
- Acknowledgements
- Discussion

1. Shaping IR Spectroscopy into a Powerful Tool for Biopharma Characterizations.
   Dipanwita Batabyal, Libo Wang , Jeffrey Zonderman, and Mats Wikström
   BioPharm International, 2020, 33, 5, 42
2. Advancing secondary structure characterization of monoclonal antibodies using Microfluidic Modulation Spectroscopy.
   Valerie A. Ivancic, Holly L. Lombardo, Eugene Ma, Mats Wikström and Dipanwita Batabyal
   Analytical Biochemistry, 2022, 646, 114629
3. Advancement of Microfluidic Modulation Spectroscopy as a Highly Sensitive Protein Characterization Technique (manuscript in review)
   Tianhui Maria Ma, Richard H. Huang, Valerie I. Collins, Mats Wikström and Dipanwita Batabyal

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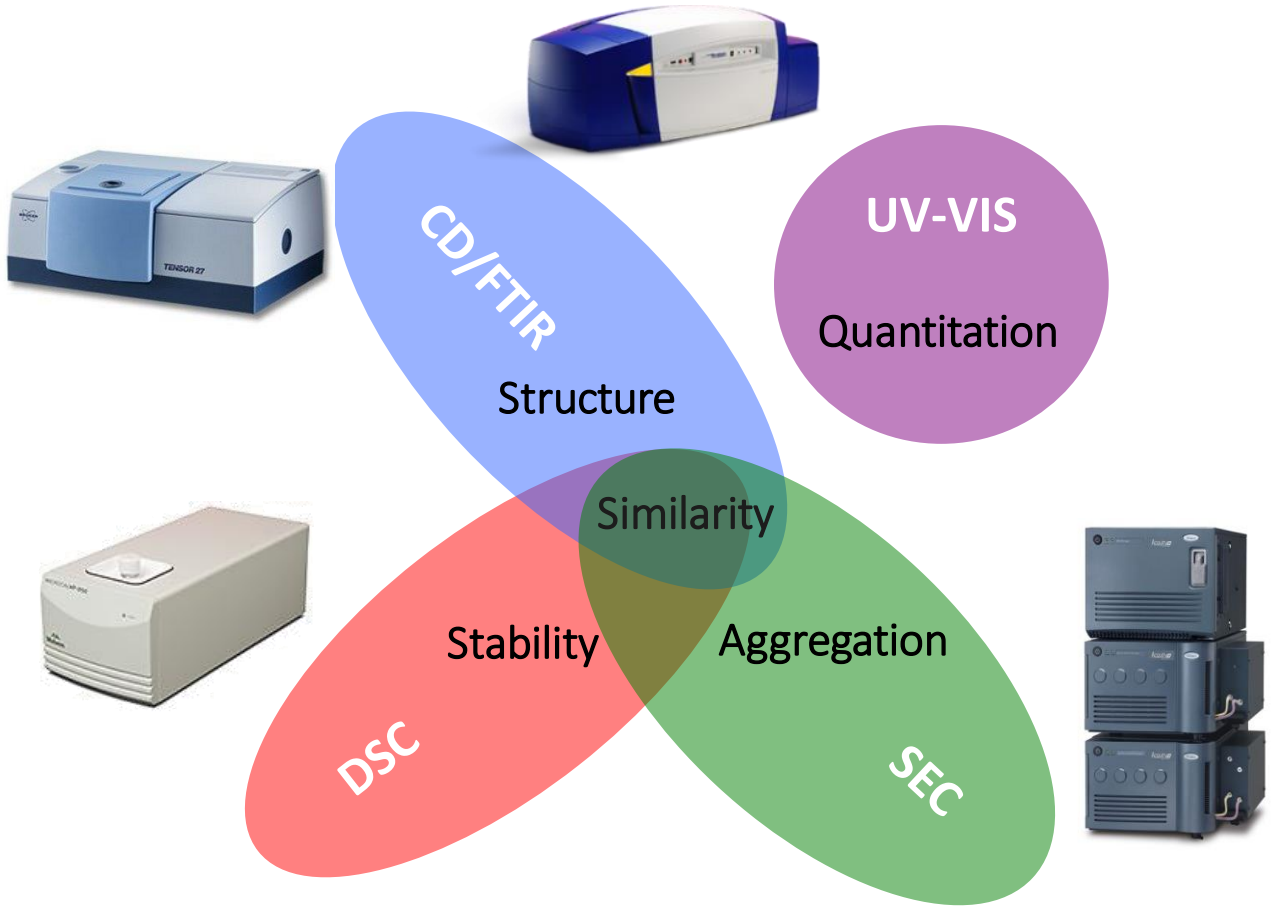
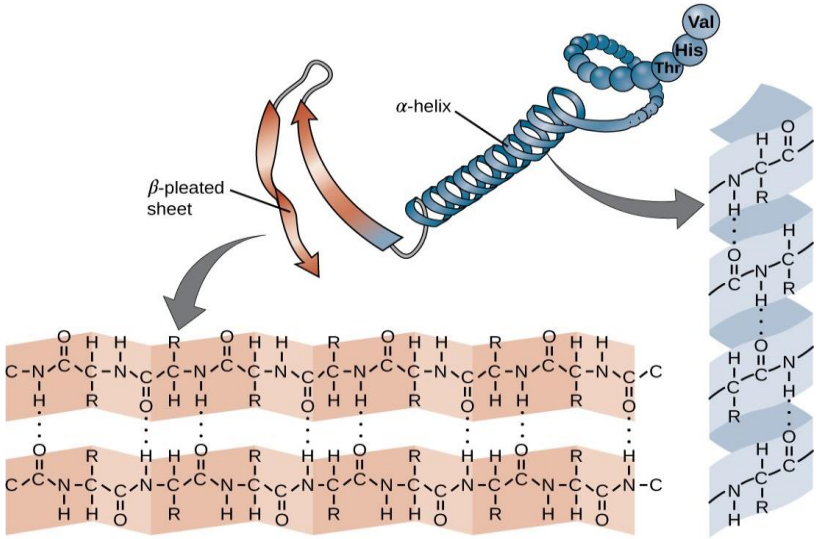
Limitations Of Today's Tools



- Narrow concentration ranges
- Limited sensitivity for detecting change
- Limited characterization per platform
- Complex workflows

Secondary Structure Characterization Tools

- **Infrared (IR) spectroscopy** has been long recognized as an extremely powerful tool for secondary structure characterization of proteins.



- Unable to be fully exploited due the current state of infrared measurement technology.
- **Traditional FTIR** – needs high sample concentrations; background drifts; significant **MANUAL** sample handling and instrument prep.
- **Far UV CD** – only works with low concentrations, susceptible to excipients; buffer exchange may be required.

How about an automated IR method with high sensitivity and reliability?

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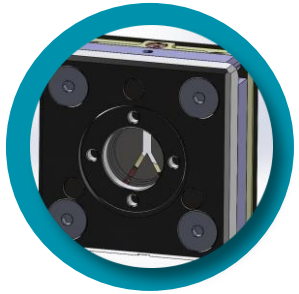
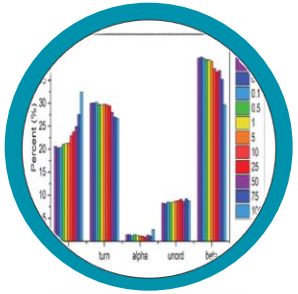
What is Microfluidic Modulation Spectroscopy (MMS)?

Sample solution and a matching buffer are automatically introduced into a microfluidic flow cell, the two fluids are rapidly modulated (e.g. 1-5 Hz) across the laser beam path to produce nearly drift-free background compensated measurements.

*QCL**

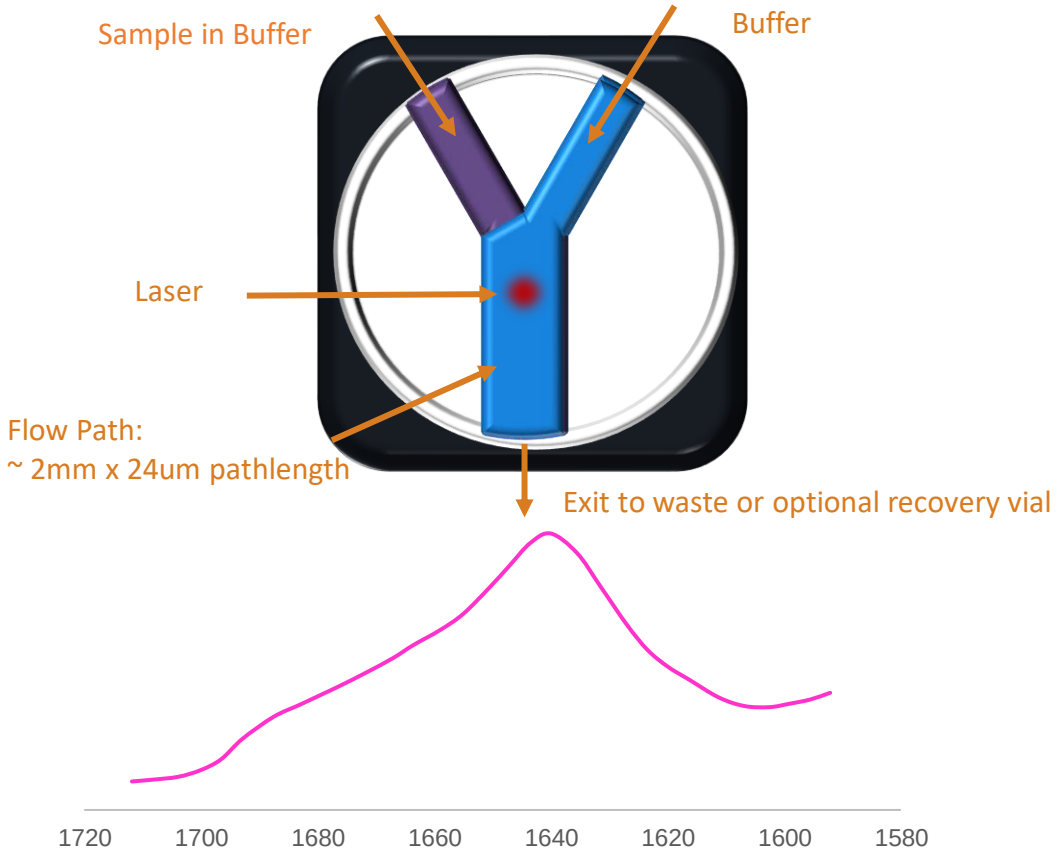


Microfluidic Flow Cell



Advanced Analytics

Automated Sample Handling System



Automatic and continuous background referencing greatly improves sensitivity while simplifying instrument workflow and data analysis.

**QCL: Quantum cascade laser*

Results from Method Testing - BETA instrument

Shaping IR Spectroscopy into a Powerful Tool for Biopharma Characterizations
Dipanwita Batabyal, Libo Wang, Jeffrey Zonderman, and Mats Wikström
BioPharm International, 2020, 33, 5, 42

In this study, we compared the MMS results with the conventional Fourier transform infrared data and presented a series of experimental studies showcasing the performance of MMS in the secondary structure characterization of biotherapeutic proteins.

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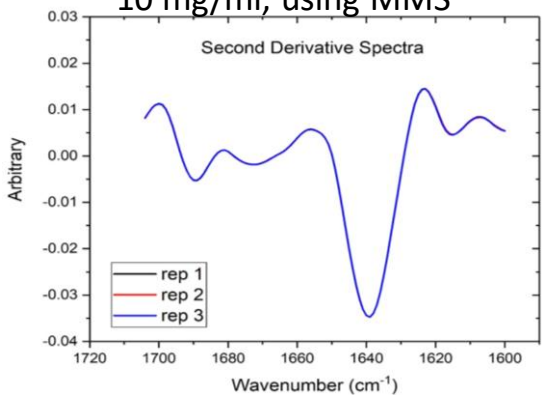
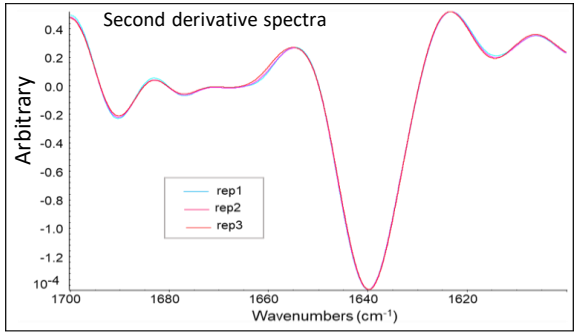
MMS And Traditional FTIR Data Are Highly Comparable

10 mg/ml, using MMS



10 mg/ml, using Bruker Vertex 70

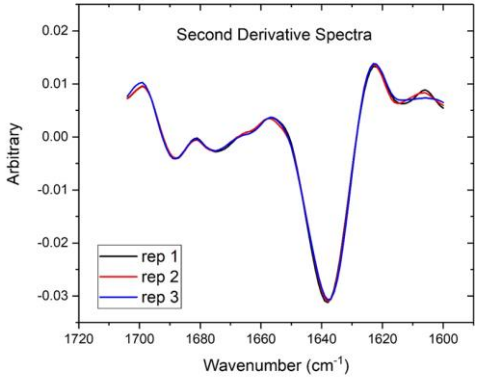


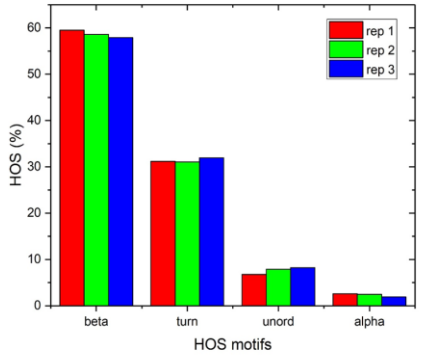
MMS can be used at low concentration (1mg/mL) with high sensitivity (similarity scores >98%)

BiTE® molecule at 1 mg/mL

Sample conc. (mg/ml)	Similarity (%) of replicates			Mean±SD
*1.0	99.01	99.20	98.97	99.06±0.12

Similarity score is calculated by comparing the area of overlap (AO) and the mean of the three runs as used as reference.





Sample conc.	HOS% (Mean±SD) of replicates			
	Beta	Turn	Unordered	Alpha
1.0mg/ml	58.67±0.80	31.38±0.48	7.64±0.78	2.32±0.36

Acceptable quality data (similarity score >95%) is not achieved at 1mg/ml using our traditional FTIR instrument.

Batabyal et al. BioPharm International, 2020, 33, 5, 42
Wen et al. Journal of Pharmaceutical Sciences, 2020,109.1,247-253.

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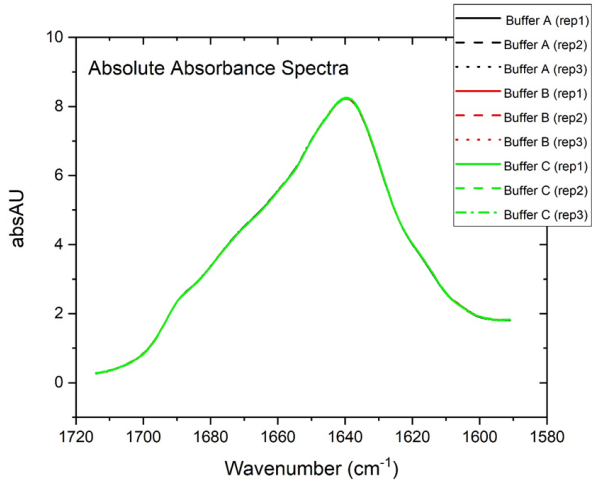
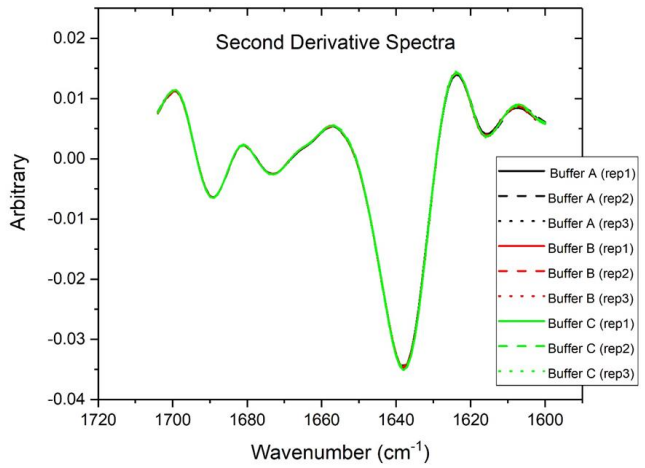
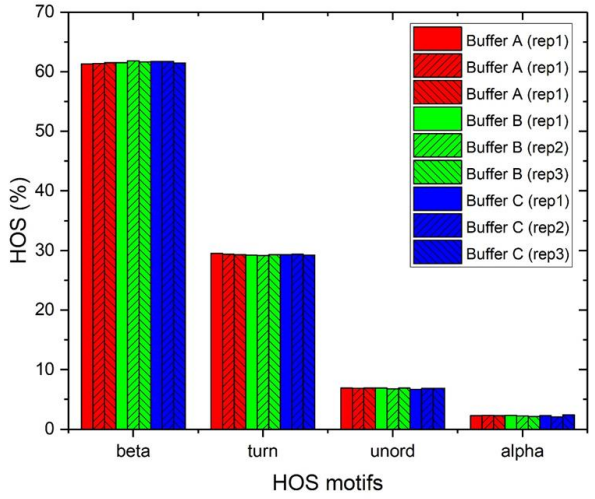
Effect of excipient (PS 80) on secondary structure using MMS

Does PS 80 (at 0.01%, 0.05% and 0.1% w/v) in buffer affect the secondary structure of a mAb (IgG2) sample (5 mg/ml)?







Samples in	Similarity (%) of replicates			Mean±SD
*Buffer A	99.62	99.79	99.76	99.72±0.09
Buffer B	99.76	99.64	99.52	99.64±0.12
Buffer C	99.66	99.55	99.69	99.63±0.07

**Similarity score is calculated by comparing the area of overlap and the mean of Buffer A runs is used as reference. Buffers A,B,C contain 0.01%, 0.05% and 0.1% w/v PS 80, respectively, and otherwise same.*

Samples in	HOS% (Mean±SD) of replicates			
	Beta	Turn	Unordered	Alpha
Buffer A	61.41±0.09	29.40±0.11	6.90±0.01	2.29±0.03
Buffer B	61.67±0.15	29.22±0.06	6.87±0.10	2.24±0.10
Buffer C	61.63±0.13	29.29±0.07	6.80±0.10	2.27±0.15

PS 80 has no effect on the secondary structure

Batabyal et al. BioPharm International, 2020, 33, 5, 42

Effect of excipient (PS 80) on secondary structure using MMS

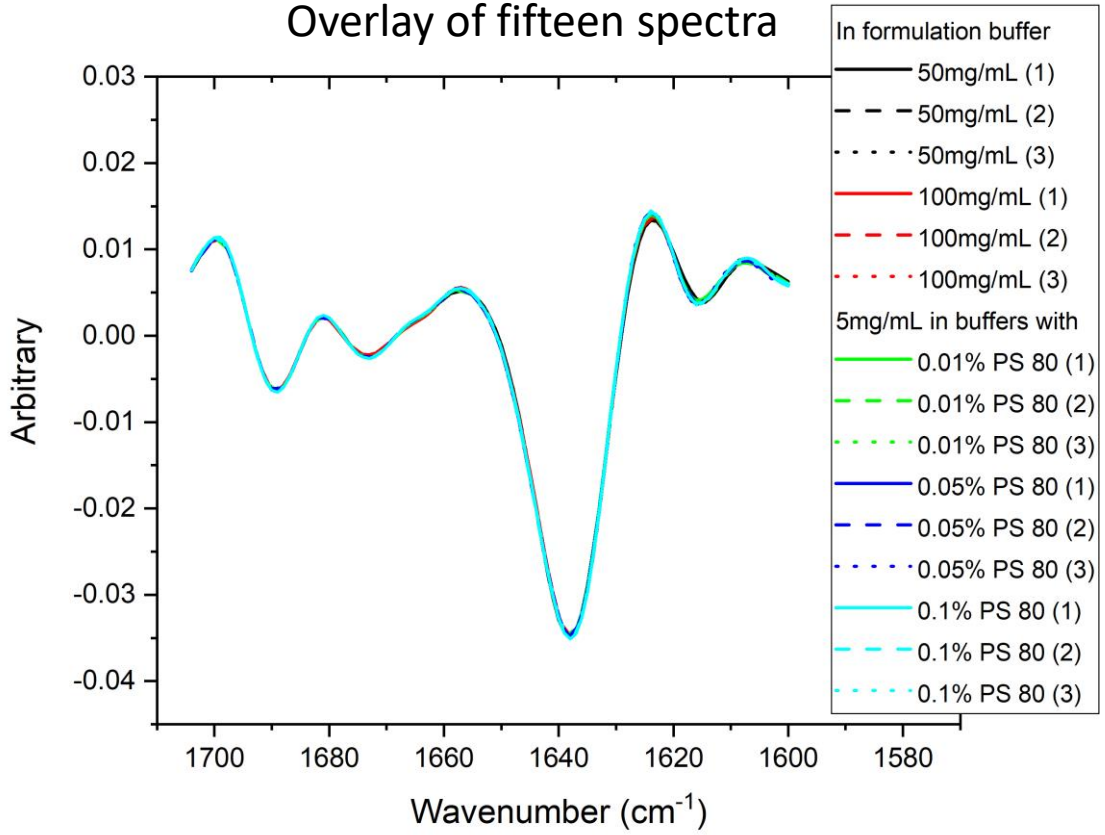
Check for Consistency And Reproducibility Of MMS Data Across Different Data Sets

Similarity score from MMS

Sample Conc.	In Buffers w/o PS 80	Similarity (%) of replicates			Mean±SD
*50 mg/ml	Base Buffer (no PS 80)	99.45	99.75	99.60	99.60±0.15
100 mg/ml	Base Buffer (no PS 80)	99.37	99.36	99.37	99.37±0.01
5 mg/ml	Buffer A (0.01% PS 80)	99.30	99.31	99.17	99.26±0.08
5 mg/ml	Buffer B (0.05% PS 80)	99.18	99.28	99.06	99.17±0.11
5 mg/ml	Buffer C (0.1% PS 80)	99.36	99.26	99.23	99.28±0.07

Similarity score is calculated by comparing the area of overlap and the mean of 50 mg/ml runs is used as reference. Buffers differ in concentrations of PS 80 only as indicated and otherwise same.

Overlay of fifteen spectra



High Consistency And Reproducibility Of MMS Data Across Different Data Sets were Observed.

Batabyal et al. BioPharm International, 2020, 33, 5, 42

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Results from Method Development - AQS3 version

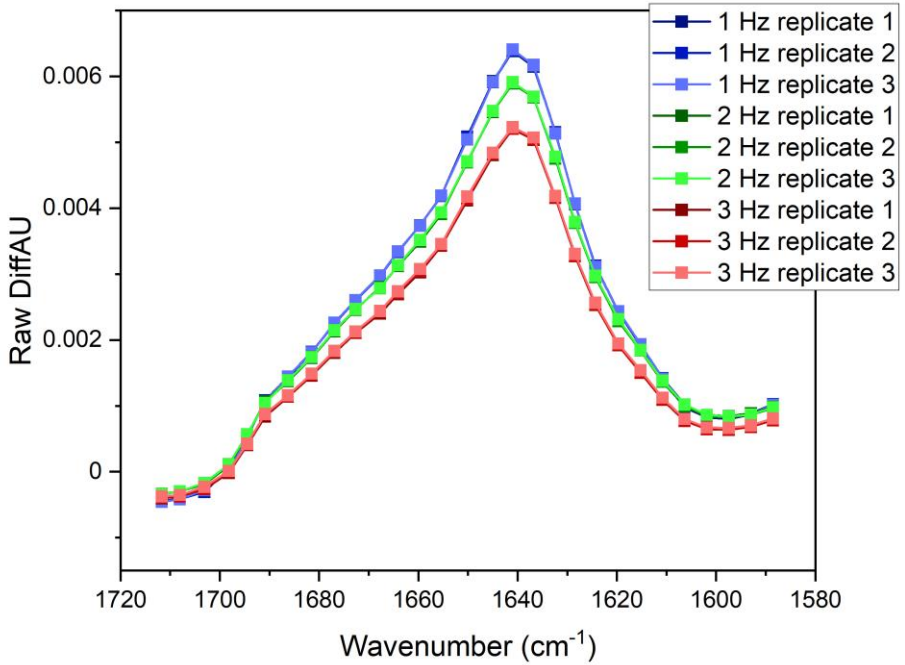
Advancing secondary structure characterization of monoclonal antibodies using Microfluidic Modulation Spectroscopy.
Valerie A. Ivancic, Holly L. Lombardo, Eugene Ma, Mats Wikström and Dipanwita Batabyal
Analytical Biochemistry, 2022, 646, 114629

In this study, monoclonal antibodies (mAbs) at concentrations ranging from 0.5 to 50 mg/mL were analyzed and high-quality data was obtained by optimizing two critical acquisition parameters (a) **sample modulation frequency** and (b) **detector dwell time settings.**

What is the effect of varying Modulation Rate?

What is a modulation frequency?

- Modulation rate is the frequency at which the spectra are collected, default modulation rate is 1 Hz, which collects data once per second.
- Here 1, 2, and 3 Hz were applied to examine the effect on data quality and overall signal-to-noise for the 1 mg/mL IgG1 sample.



The raw differential absorbance spectra of 1.0 mg/mL IgG1 at 1, 2, and 3 Hz modulation rates showing the effect of increasing the modulation rate above that necessary for acceptable lamellar flow. The overall signal decreases as the modulation rate increases due to mixing in the cell.

NOTE: Modulation Auto Discovery setting can be used to automatically allow determination of the optimal sample modulation setting during the Fluid Entry step

Ivancic et al. Analytical Biochemistry, 2022, 646, 114629

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What is the effect of varying Test Sequence?

What is a test sequence?

A combination of 4 wait/dwell times when the detector shutter is opened and closed during data collection.

Sequence Values	Step	Sequence Name and Seconds per Step			
		223**5**	223**9**	223**16**	243**16**
First Digit	Dark Wait	2	2	2	2
Second Digit	Dark Dwell*	2	2	2	4
Third Digit	Light Wait	3	3	3	3
Fourth Digit (s)	Light Dwell*	5	9	16	16

**The dwell times are configurable in the software and were varied in this study.*

Dark wait: the laser is given a chance to settle at the new wavenumber position

*Dark dwell: data is collected with the shutter closed to obtain the dark offset noise

Light wait: when the shutter opens, and flow is established in the flow cell

***Light dwell: data is collected for the sample and its reference buffer**

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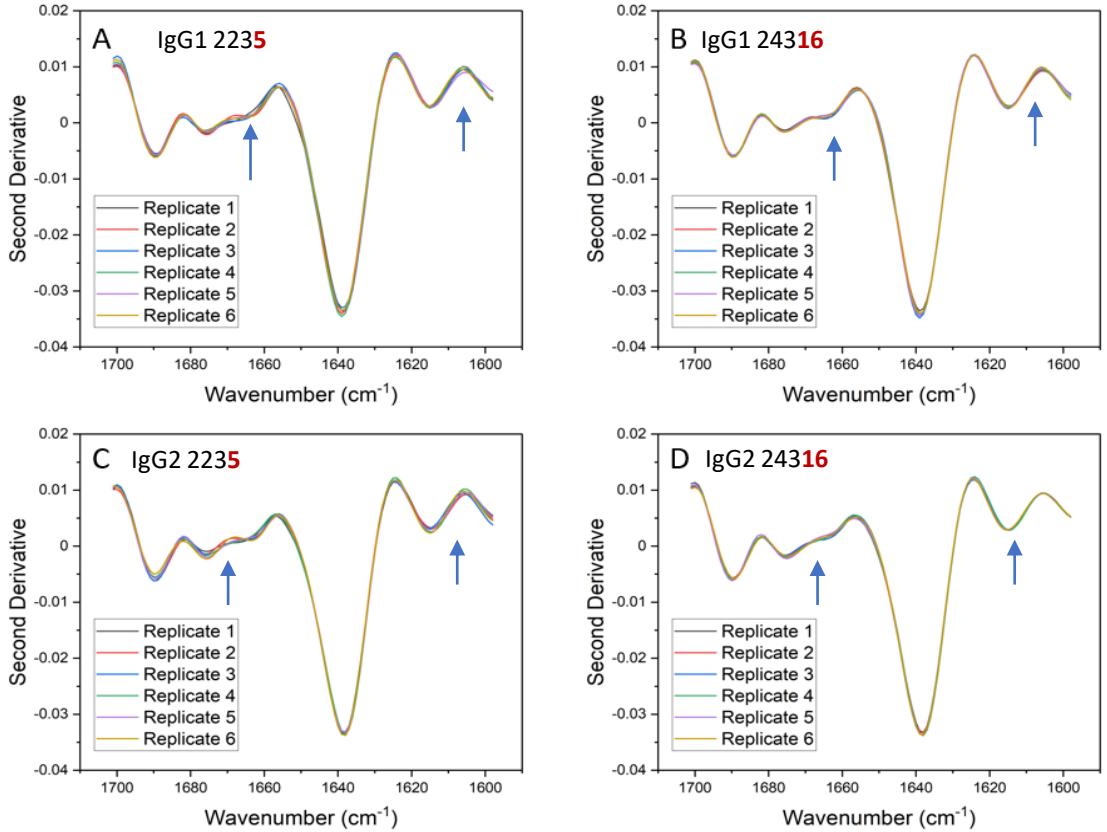
Similarity scores for IgG1 and IgG2 vs concentration

This work gives us the ability to compare data quality relative to test sequence settings and aids in selecting optimal test conditions for specific concentration.



Percent similarity scores for all concentrations of IgG1 and IgG2 using 4 different test sequences.

mAb	Test Sequence	Sample Concentrations (mg/mL)							
		0.5	0.7	1	1.5	2	3	10	50
IgG1	2235	97.57	98.37	98.70	99.26	99.47	99.50	99.84	99.98
	2239	98.00	98.77	99.09	99.25	99.55	99.77	NA	NA
	22316	98.40	98.94	99.23	99.39	NA	NA		
	24316	98.48	98.90	99.25	99.41	99.67	99.76		
IgG2	2235	97.63	98.51	98.86	99.22	99.59	99.76	99.91	99.98
	2239	98.13	98.85	99.16	99.37	99.60	99.68	NA	NA
	22316	98.42	98.88	99.31	99.59	NA	NA		
	24316	98.61	98.93	99.25	99.46	99.73	99.72		

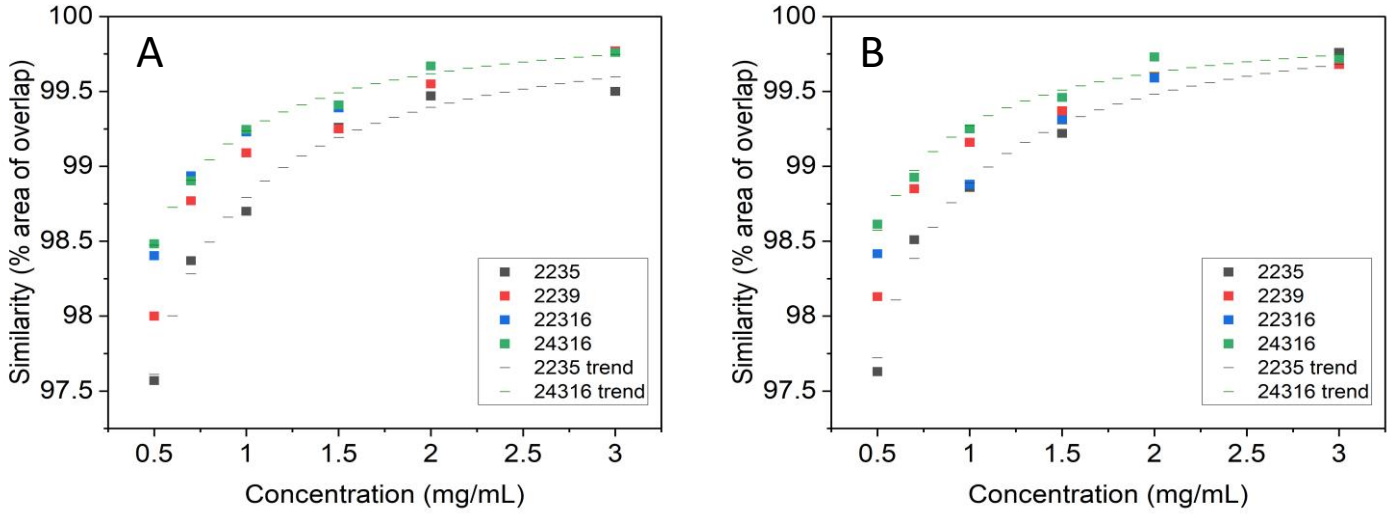
Six replicates were averaged for samples < 2 mg/mL and 3 replicates were averaged for samples ≥ 2 mg/mL.

IgG1 (A) and IgG2 (C) at 1 mg/mL were tested using the default 2235 test sequence and show 98.70% and 98.86% similarity, respectively, compared to the overall average of the replicates. 1 mg/mL IgG1 (B) and IgG2 (D) were also tested using the optimized 24316 sequence and both show 99.25% similarity compared to the overall average of the replicates.

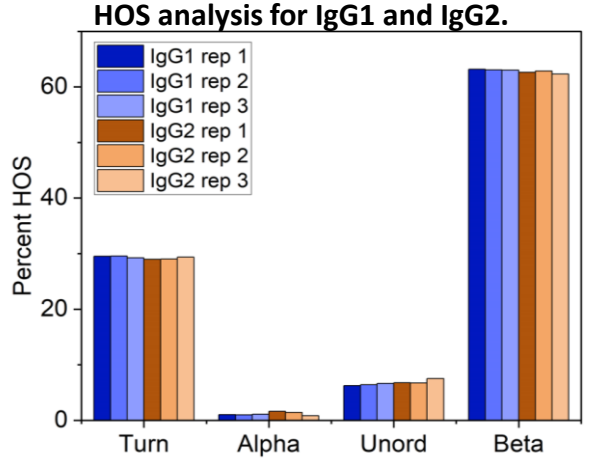
Ivancic et al. Analytical Biochemistry, 2022, 646, 114629

Similarity scores for IgG1 and IgG2 vs Concentration

IgG1 (A) and IgG2 (B) similarity scores are dependent on sample concentration and test sequence. This effect is more pronounced for low concentrations.



HOS analysis for IgG1 and IgG2.



	HOS			
	beta	**unordered**	**alpha**	**turn**
IgG1, 1 mg/mL	63.3 +/- 0.78	6.3 +/- 0.30	0.9 +/- 0.17	29.4 +/- 0.29
IgG1, 3 mg/mL	63.1 +/- 0.08	6.4 +/- 0.20	1.0 +/- 0.05	29.4 +/- 0.18
IgG1, 10 mg/mL	62.9 +/- 0.14	6.8 +/- 0.16	1.0 +/- 0.11	29.4 +/- 0.19
IgG2, 1 mg/mL	62.4 +/- 0.50	7.0 +/- 0.53	1.6 +/- 0.27	29.1 +/- 0.23
IgG2, 3 mg/mL	62.6 +/- 0.28	7.0 +/- 0.44	1.3 +/- 0.41	29.1 +/- 0.21
IgG2, 10 mg/mL	62.1 +/- 0.06	7.1 +/- 0.30	1.5 +/- 0.16	29.3 +/- 0.08

This data demonstrates the ability to compare data quality relative to test sequence settings and in selecting optimal test conditions for specific applications.

Analytical Biochemistry, 2022, 646, 114629

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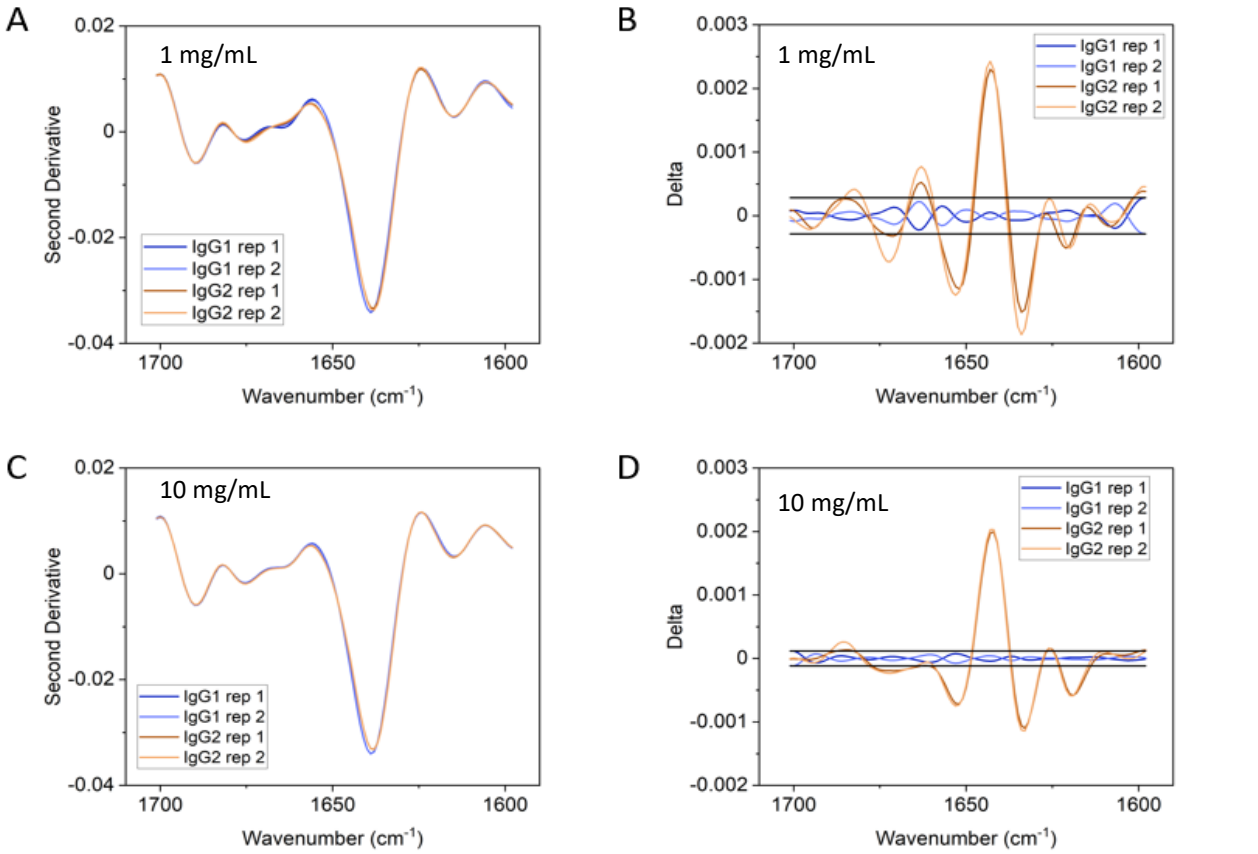
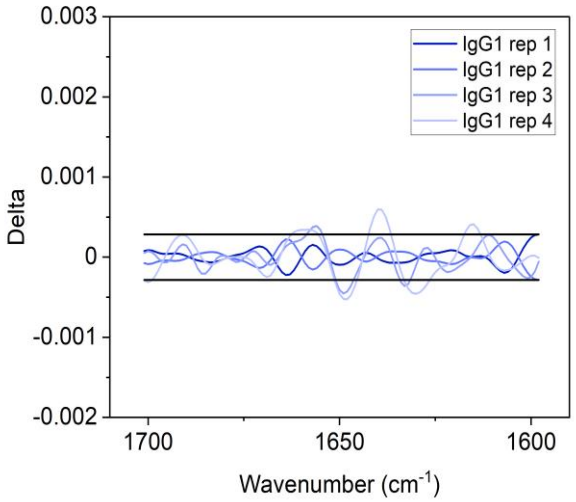
Can MMS pick up subtle differences?

The difference in IgG1 and IgG2 is subtle and does not lead to any significant changes in the overall secondary structure, however, they are detectable.



IgG1 and IgG2 at 1 mg/mL second derivative (A) and delta plot (B). IgG1 and IgG2 at 10 mg/mL second derivative (C) and delta plot (D). The delta plots highlight the differences between the second derivative plots by subtracting the replicates for IgG1 from itself and from IgG2. The horizontal lines represent the replicate-to-replicate differences.



The delta plot of 1 mg/mL IgG1 using replicates 1 and 2 as the reference control compared to replicates 3 and 4 of IgG1 shows no distinct difference

Ivancic et al. Analytical Biochemistry, 2022, 646, 114629

Wen et al. Journal of Pharmaceutical Sciences, 2020,109.1,247-253.

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Results from Method Advancement - Apollo/AQS3

Advancement of Microfluidic Modulation Spectroscopy as a Highly Sensitive Protein Characterization Technique (manuscript in review)

Tianhui Maria Ma, Richard H. Huang, Valerie I. Collins, Mats Wikström and Dipanwita Batabyal

- This study evaluates and compares the different well plate formats and scan modes of two MMS instruments.
- The newer Apollo system features a high throughput 96-well plate format and sweep scan mode that allows a 50% reduction in sample volume consumption and measurement time compared to the previous system.
- The limit of quantitation (LOQ) for determining structural impurity using the sweep scan mode was also evaluated using low protein concentration (2mg/mL).

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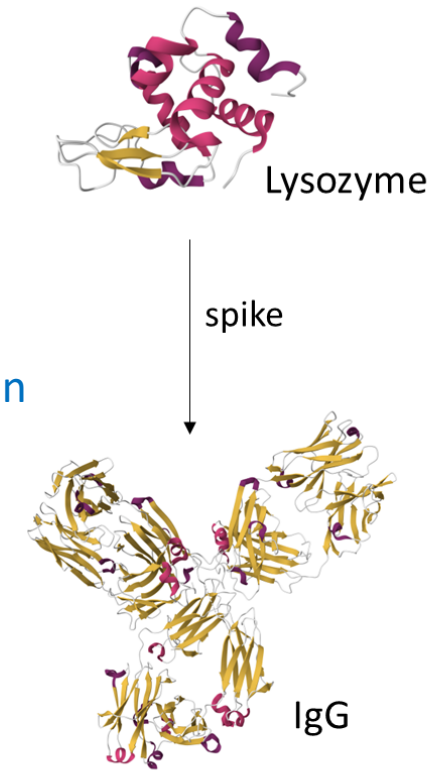
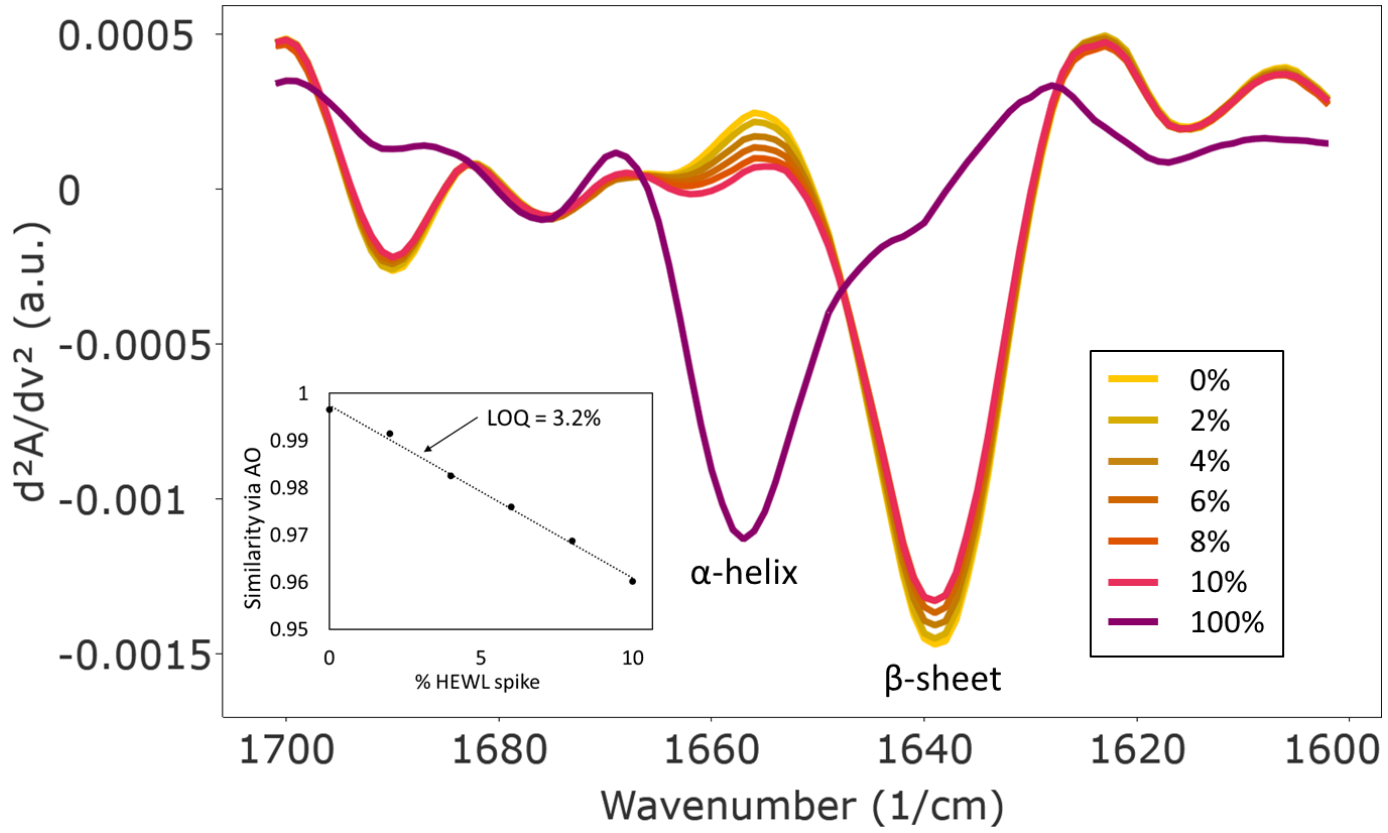
Comparing different scan modes and well plate formats

- 24 vs 96 well plate
- Step vs Sweep scan
- LOQ using Sweep scan





Spectra of IgG1 with 0%, 2%, 4%, 6%, 8%, 10% and 100% HEWL. LOQ is determined to be 3.2% for 2 mg/mL

Precision remained the same between plate formats and scan modes.

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Conclusions

- MMS represent a valuable automated IR technology for the generation of highly reliable protein secondary structure data

- The ability to optimize the settings affords additional opportunity to the user to improve signal and evaluate the secondary structure for low concentration biotherapeutic formulations and modalities.

- Performance remained the same between different plate formats, as well as between the step and sweep scan modes. The LOQ for determining structural impurity was 3.2% using a 2 mg/mL protein concentration.

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